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Aspirin provides cyclin-dependent kinase 5-dependent protection against subsequent hypoxia/reoxygenation damage in culture

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Abstract

Aspirin [acetylsalicylic acid (ASA)] is an anti-inflammatory drug that protects against cellular injury by inhibiting cyclooxygenases (COX), inducible nitric oxide synthase (iNOS) and p44/42 mitogen-activated protein kinase (p44/42 MAPK), or by preventing translocation of nuclear factor κB (NF-κB). We studied the effect of ASA pre-treatment on neuronal survival after hypoxia/reoxygenation damage in rat spinal cord (SC) cultures. In this injury model, COX, iNOS and NF-κB played no role in the early neuronal death. A 20-h treatment with 3 mM ASA prior to hypoxia/reoxygenation blocked the hypoxia/reoxygenation-induced lactate dehydrogenase (LDH) release from neurons. This neuroprotection was associated with increased phosphorylation of neurofilaments, which are substrates of p44/42 MAPK and cyclin-dependent kinase 5 (Cdk5). PD90859, a p44/42 MAPK inhibitor, had no effect on ASA-induced tolerance, but olomoucine and roscovitine, Cdk5 inhibitors, reduced ASA neuroprotection. Hypoxia/reoxygenation alone reduced both the protein amount and activity of Cdk5, and this reduction was inhibited by pre-treatment with ASA. Moreover, the protein amount of a neuronal Cdk5 activator, p35, recovered after reoxygenation only in ASA-treated samples. The prevention of the loss in Cdk5 activity during reoxygenation was crucial for ASA-induced protection, because co-administration of Cdk5 inhibitors at the onset of reoxygenation abolished the protection. In conclusion, pre-treatment with ASA induces tolerance against hypoxia/reoxygenation damage in spinal cord cultures by restoring Cdk5 and p35 protein expression.

Keywords: aspirin, cyclin-dependent kinase 5, mitogen-activated protein kinase.

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Aspirin [acetylsalicylic acid (ASA)], a widely used non-steroidal anti-inflammatory drug, has been recently found to be neuroprotective in experimental models of focal brain ischemia (Khayyam *et al*. 1999), amyotrophic lateral sclerosis (Barneoud and Curet 1999), Parkinson's disease (Teismann and Ferger 2001), *in vitro* models of hypoxic retinal (Maynard *et al*. 1998) and hippocampal injury (Riepe *et al*. 1997), and in brain slice and culture models of excitotoxicity (Grilli *et al*. 1996). ASA is known to be a non-selective inhibitor of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), but also displays various other potentially beneficial effects, such as inhibition of a proinflammatory enzyme, inducible nitric oxide synthase (iNOS) (Amin *et al*. 1995), and preventing nuclear translocation of the reactive oxygen species-sensitive transcription factor NF-κB (Yin *et al*. 1998). Moreover, salicylates have been shown to inhibit the activity of extracellular-signal regulated kinases (ERKs) in neutrophils (Pillinger *et al*. 1998). Because of these multiple, potentially beneficial effects of ASA, the mechanism of how ASA provides neuroprotection is not well known.

We have previously used a rat spinal cord (SC) culture model to study the effects of inhibitors of COX enzymes piroxicam and NS-398 on neuronal survival after hypoxia/reoxygenation insult (Vartiainen *et al*. 2001). In this hypoxia/reoxygenation model, both of the COX inhibitors were neuroprotective, even though no measurable levels of COX or iNOS enzymes were present, and inhibition of the binding activity of NF-κB was not associated with the neuroprotection. Instead, modulation of ERKs and/or some other

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Abbreviations used: ASA, acetylsalicylic acid; Cdk5, cyclin-dependent kinase 5; COX, cyclooxygenase; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; LDH, lactate dehydrogenase; NF, neurofilament; p44/42 MAPK, p44/42 mitogen-activated protein kinase.

kinase pathways may mediate the beneficial effects of ASA (N. Vartiainen *et al.* unpublished).

Here we show that the presence of ASA in the culture medium during exposure to hypoxia/reoxygenation is not required for ASA-induced neuroprotection. Instead, neuroprotection can be achieved by pre-treatment with 3 mmol/L ASA for 20 h. We also show that this ASA-induced tolerance is dependent on the activity of cyclin-dependent kinase 5 (Cdk5), which is increased by ASA pre-treatment compared with non-treated cultures during early reoxygenation.

Experimental procedures

Cell culture

SC cultures were prepared as described previously (Vartiainen *et al.* 1999). Briefly, SCs were dissected from E14 rat embryos (Wistar; University of Kuopio). The tissue was trypsinized for 15 min [0.25% trypsin–EDTA in Dulbecco's modified Eagle's medium (DMEM)] and centrifuged for 5 min at 280 *g*. The supernatant was removed and the tissue triturated using a fire-polished Pasteur pipette to achieve a single-cell suspension. The cell solution was diluted to the desired density and plated on poly L-lysine-coated cell culture plates in high-glucose DMEM with 10 μg/mL gentamycin, supplemented with 10% fetal bovine serum (FBS) and 10% heat-inactivated horse serum (HS). The following day the culture medium was replaced with DMEM supplemented with 5% of both sera. After 4 days of culture *in vitro*, the medium was replaced with DMEM supplemented with 5% of both sera and 5 μmol/L cytosine arabinoside (AraC), and then replaced with DMEM containing 5% HS on the following day.

Drug treatments

ASA (Sigma, St Louis, MO, USA) at a concentration of 3 mM was used in the present study, as according to our preliminary studies (N. Vartiainen *et al.* unpublished), this is the most effective dose against hypoxia/reoxygenation damage [according to release of the cell death marker lactate dehydrogenase (LDH) and the total cell count], and 3 mM serum levels of ASA can be easily reached in humans (Weissmann 1991). PD98059 (20 μmol/L), a selective inhibitor of p44/42 MAP kinase activation, was purchased from Tocris Cookson (Ballwin, MO, USA). Olomoucine (50 μmol/L), a non-selective Cdk5 inhibitor (Vesely *et al.* 1994) and roscovitine (20 μmol/L), a selective Cdk5 inhibitor (Meijer *et al.* 1997), were purchased from Sigma and Tocris Cookson, respectively. All compounds were dissolved in dimethylsulfoxide (DMSO), and appropriate DMSO controls were used. For pre-treatments the drugs were administered to 6-day cultures *in vitro* in DMEM + 5% HS for 24 h. At 7 days *in vitro* the medium was replaced with control medium containing DMEM + 1% HS, but no drugs. In another set of experiments, olomoucine (50 μmol/L) or roscovitine (20 μmol/L) were administered in ASA pre-treated cultures at the onset of reoxygenation.

Hypoxia treatment

Fifteen to 30 min after replacing the pre-treatment drugs with control medium the cultures were exposed to hypoxia. This was induced by placing the plates in an airtight chamber that was flushed with 5% CO_2/95% N_2 for 10 min to achieve a low-oxygen environment (Yu *et al.* 1989). The chamber was placed in a 37°C incubator for 20 h. After the hypoxia period, the chamber was opened and the plates were placed in a normoxic incubator for 0–4 h.

Assessment of cell death

In this hypoxia/reoxygenation model, neuronal cell death increases after 2 h of reoxygenation and is significant after 4 h of reoxygenation (Vartiainen *et al.* 2001). After 4 h of reoxygenation the culture media were collected for measurement of LDH release. We have previously shown that the LDH release correlates well with neuronal cell loss in this model (Vartiainen *et al.* 2001). Determination of LDH release was performed spectrophotometrically at 340 nm using Sigma LDH-measurement reagent. The statistical comparison was performed using ANOVA.

Immunocytochemistry

The cells were fixed with 4% paraformaldehyde, permeabilized and non-specific binding was blocked by incubation with 2% bovine serum albumin (BSA) in 0.01 mol/L phosphate-buffered saline (PBS) and 0.3% Triton-X-100 (blocking buffer). Anti-phosphorylated medium- and high-molecular weight neurofilaments primary antibody (SMI-31, 1 : 5000 dilution; Sternberger Monoclonals, Inc., Baltimore, MD, USA) was diluted in the same buffer. After incubation for 48–72 h at 4°C, the cells were washed with PBS, and the secondary antibody [fluorescein isothiocyanate (FITC)-labeled anti-mouse, 1 : 80 dilution; Jackson Immuno-Research, West Grove, PS, USA) was diluted in the blocking buffer and applied to the cells for 2 h. The density of the immunoreactive neurons, as well as the number of total cells, was assessed using a Nikon Diaphot 300 fluorescence microscope (Nikon, Europe Bv, Badhoeredorp, the Netherlands) equipped with an appropriate filter set and by counting the positively labeled cells from five fields (1.5×10^{-2} mm^2) per culture well. The results from three wells from three independent experiments were combined.

Cdk5 activity assay

Active Cdk5 was immunoprecipitated in a complex with p35. Two micrograms of rabbit polyclonal antibody against p35 and p25 (C-19, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) were pre-absorbed on 50 μL of protein G–Sepharose (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and washed with Tris-buffered saline–Tween (TBST). Thirty micrograms of protein from the pre-cleared cell lysates was added and the total volumes brought to 100 μL. Immunoprecipitation was carried out overnight at 4°C with continuous rotation of the samples. The complexes were washed twice with TBST and three times with kinase assay buffer [100 mmol/L HEPES, pH 7.0, 10 mmol/L $MgCl_2$, 10 mmol/L $MnCl_2$, 10 mmol/L EGTA, 1 mmol/L dithiothreitol (DTT), 0.1 mmol/L Na_3VO_4, 40 mmol/L beta-glycerophosphate]. Kinase assays were carried out at 37°C for 15 min in 50 μL of kinase buffer containing 100 μg/mL histone H1 (Sigma), 3 μmol/L ATP and 5 μCi [γ^{32}P]-ATP. The reaction was stopped by adding 5× sodium dodecyl sulfate (SDS) sample buffer, and 10-μL aliquots were loaded onto a 10% SDS–polyacrylamide gel. After electrophoresis, the gel was washed with 1% solution of sodium pyrophosphate in 5% tricloroacetic acid, dried and exposed

overnight to phosphoscreen. The phosphoscreen was scanned on a STORM phosphoimager (Molecular Dynamics, Sunnyvale, CA, USA) and the bands were quantified with ImageQuant software (Molecular Dynamics).

Immunoblotting

Cells grown on 35-mm plates were lysed and scraped into buffer containing 320 mmol/L sucrose, 1 mmol/L Tris–HCl, pH 7.4, 1 mmol/L EGTA, 1 mmol/L Na_3VO_4, 4 mmol/L DTT, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 0.2 mmol/L phenylmethylsulfonyl fluoride (PMSF), 50 mmol/L NaF and 0.5% Nonidet P-40 (NP-40). The samples were boiled for 5 min with 5× Laemmli sample buffer. Electrophoresis was carried out with 10 μg protein in a 10% SDS polyacrylamide gel. Separated proteins were transferred onto Hybond-P membrane (Amersham Pharmacia Biotech). Cdk5 protein was detected using anti-Cdk5 antibody (C-8; Santa Cruz Biotechnology) and p35/p25 with C19 antibody (Santa Cruz Biotechnology). Horseradish peroxidase-labeled secondary antibody and an ECL Plus kit (both Amersham Pharmacia Biotech) were used for visualization of immunoreactive bands. The membranes were directly scanned on a STORM phosphoimager (Molecular Dynamics) and the bands quantified using ImageQuant software.

Results

In the SC cultures pre-treated with 3 mmol/L ASA for 20 h and exposed to hypoxia 15–30 min later, LDH release was significantly increased after 4 h of reoxygenation when compared with non-treated cultures. When olomoucine or roscovitine, Cdk5 inhibitors, or PD98059, a p44/42 mitogen-activated protein kinase (MAPK) inhibitor, were administered to the cells together with ASA, olomoucine and roscovitine decreased the protective effect gained by ASA ($p < 0.01$), while PD98059 did not significantly change the protection (Fig. 1a). Administration of PD98059, olomousine or roscovitine alone was not toxic to the cells.

Because the inhibitor studies suggested a role of Cdk5 in ASA-induced neuroprotection, we studied phosphorylation of high- and medium-molecular-weight neurofilaments, known substrates of Cdk5 and p44/42 MAPK (Guidato *et al.* 1996; Sun *et al.* 1996). Immunocytochemistry was carried out using a phosphorylation-dependent antibody, SMI-31. In addition to neurofilament recognition, this antibody (at the dilution used in this study) cross-reacts with some nuclear, potentially histone, proteins causing a background staining that can be used to evaluate the total cell number. ASA induced accumulation of phosphorylated neurofilaments in neuronal somata (Fig. 2b; arrows) when compared with control cultures or untreated cultures exposed to hypoxia/reoxygenation (Fig. 2a). Administration of olomoucine and roscovitine, but not PD98059, decreased the number of neurons immunoreactive for phosphorylated neurofilaments when administered together with ASA (Fig. 2c, olomoucine). After 4 h of reoxygenation, the total cell number in non-treated samples was 64.5 ± 35.4, while in

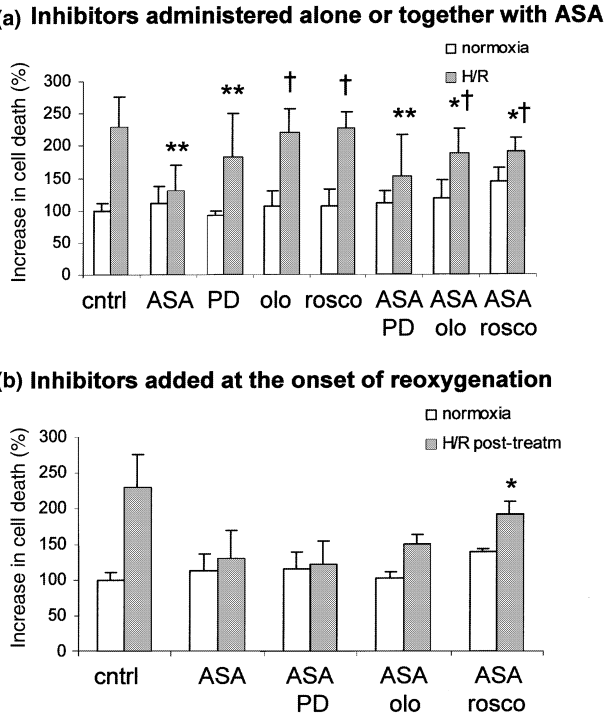

Fig. 1 Lactate dehydrogenase (LDH) release into the culture medium was measured after 24 h of normoxia, or after 20 h of hypoxia and 4 h of reoxygenation (H/R). In panel (a) the effect of pre-treatment using acetylsalicylic acid (ASA), the p44/42 mitogen-activated protein kinase (MAPK) pathway inhibitor PD98059 or cyclin-dependent kinase 5 (Cdk5) inhibitors olomoucine and roscivitine on neuronal survival was determined. Furthermore, the role of these inhibitors on the neuron protection gained by ASA pre-treatment was studied using co-treatment with ASA and the inhibitors. Cntrl, non-treated samples; PD, PD98059; olo, olomoucine; rosco, roscovitine. *$p < 0.05$; **$p < 0.01$, when compared with non-treated hypoxia/reoxygenation samples; †$p < 0.01$ when compared with ASA pre-treated samples (ANOVA). Values represent mean ± SD; $n = 15$. In panel (b) LDH measurements were performed using the ASA pre-treated samples where the kinase inhibitors were added at the start of reoxygenation. *$p < 0.05$ when compared to ASA-treated hypoxia/reoxygenation samples.

ASA pre-treated samples the cell number was 256.7 ± 37.3 ($p < 0.01$). When ASA and olomoucine were administered together, the cell number decreased to 107.0 ± 34.0 ($p < 0.01$, ANOVA; data combined from three different experiments, $n = 3$ in each experiment).

As active Cdk5 seemed to be important for neuronal survival, we studied whether the kinase activity during the reoxygenation phase was affected by hypoxia/reoxygenation and/or ASA, thus contributing to the neuronal survival assessed by LDH release. The inhibitors were added to ASA pre-treated cells at the start of reoxygenation, and the cellular survival was measured 4 h later (Fig. 1b). Post-treatment with PD98059 did not influence the ASA neuroprotection. However, olomoucine had a tendency to decrease cellular

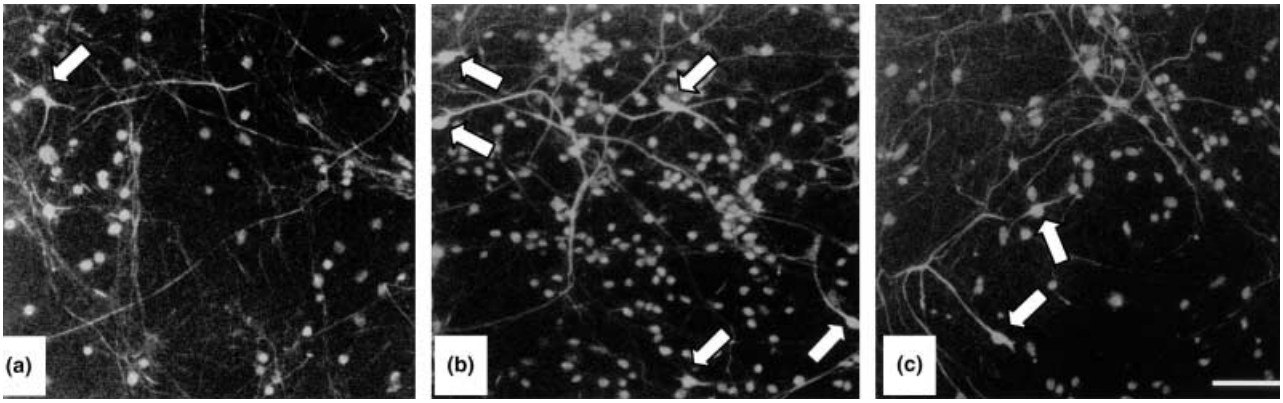

Fig. 2 Photomicrographs of immunocytochemically detected phosphorylated neurofilaments (p-NF). (a) Non-treated sample after hypoxia and 4 h of reoxygenation, (b) acetylsalicylic acid (ASA) pre-treatment, (c) olomoucine administered together with ASA. Evaluation of the cellular survival was performed by counting the total number of cells. Scale bar 50 μm. Arrows, neuronal somata with p-NF-immunoreactivity.

survival, and roscovitine, which is a more selective and potent Cdk5 inhibitor than olomoucine, significantly ($p < 0.05$) decreased the cellular survival when administered to the cells for the reoxygenation period. These results support the idea that during the reoxygenation phase activity of Cdk5, but not p44/42 MAPK, is crucial for ASA-induced tolerance.

We next studied, using a gel kinase activity assay, whether the Cdk5 kinase activity was altered by these treatments. In normoxia, no difference between non-treated and ASA-treated samples was detected (Figs 3a and b). Interestingly, there was a dramatic decrease down to 40% of the original level ($p < 0.01$) in the Cdk5 kinase activity after hypoxia in both non-treated and ASA-treated samples. After 2 h of reoxygenation, when the cell death was not significant, the kinase activity in non-treated samples showed a further decrease to 24% of the original level, while the activity in ASA-treated samples was restored to 48% of the original, being significantly higher than after hypoxia alone ($p < 0.01$).

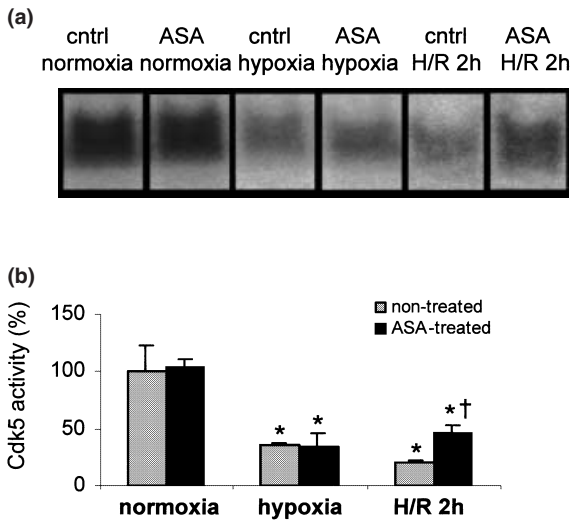

Fig. 3 The kinase activity of cyclin-dependent kinase 5 (Cdk5) was assayed using immunoprecipitated samples. Cntrl, non-treated; H/R 2 h, hypoxia followed by 2 h of reoxygenation. (a) Representative kinase activity gels. (b) Quantified values of the kinase activity assay. *$p < 0.01$ when compared with the normoxic non-treated samples; †$p < 0.01$ when compared with hypoxia/reoxygenation non-treated samples (ANOVA). Values represent mean ± SD; $n = 3$.

As the Cdk5 kinase activity was decreased by hypoxia and hypoxia/reoxygenation, we studied the protein expressions of Cdk5 and p35 (a major neuronally expressed Cdk5 activator) in the SC cultures in normoxia, after 20 h of hypoxia and after 15 min and 2 h of reoxygenation. In normoxic cultures no significant difference was detected in Cdk5 or p35 protein expression between non-treated and ASA-treated samples (Figs 4a and b, and Figs 5a and b). Hypoxia caused a significant decrease in the level of Cdk5 protein in non-treated samples ($p < 0.05$), but not in ASA-treated samples (Fig. 4). After hypoxia the level of p35 protein was significantly decreased in both non-treated and ASA-treated samples (Fig. 5). After 15 min of reoxygenation there was no significant change in the protein levels when compared with hypoxia alone (data not shown), but after 2 h of reoxygenation the Cdk5 protein level had decreased further to 24% of the original level in non-treated samples (Fig. 4b, $p < 0.01$ when compared with non-treated normoxic samples). However, ASA treatment restored the protein expression to 50% of that of the control, and the expression was significantly higher than in non-treated hypoxia/reoxygenation samples (Fig. 4b, $p < 0.01$). A similar pattern was seen in p35 protein expression after reoxygenation (Fig. 5, $p < 0.01$). No detectable cleavage of p35 to p25 was detected at the time-points used in this study (data not shown).

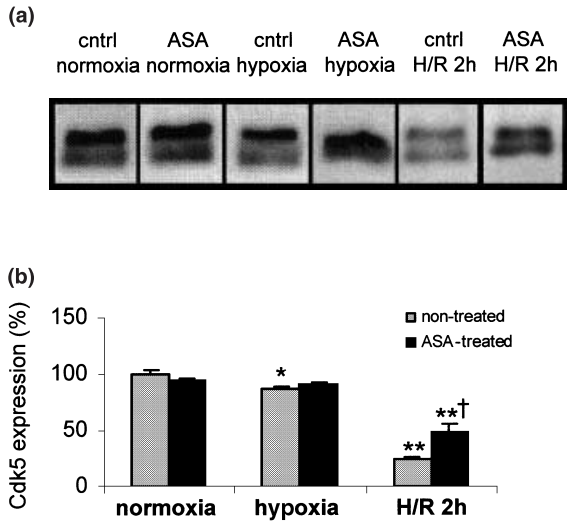

Fig. 4 Immunoblotting with a cyclin-dependent kinase 5 (Cdk5) antibody was performed to study the effect of acetylsalicylic acid (ASA) pre-treatment on Cdk5 protein expression. Cntrl, non-treated; H/R 2 h, hypoxia followed by 2 h of reoxygenation. (a) Representative immunoblots. (b) Quantification data of Cdk5 protein. $*p < 0.05$.; $**p < 0.01$, when compared with normoxic non-treated samples, respectively; $\dagger p < 0.01$ when compared with hypoxia/reoxygenation non-treated sample (ANOVA). Values represent mean ± SD; $n = 3$.

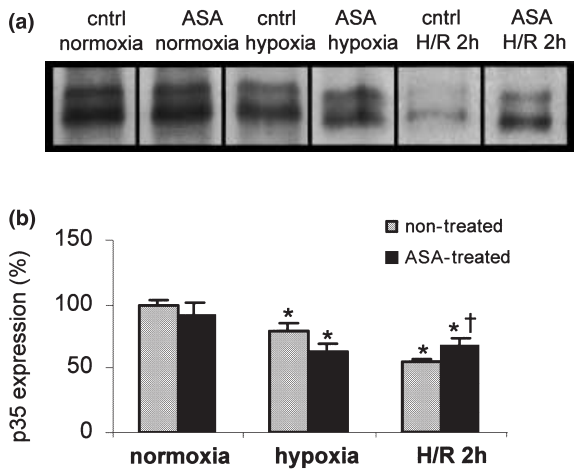

Fig. 5 Immunoblotting with a p35 antibody was carried out to study the effect of acetylsalicylic acid (ASA) pre-treatment on p35 protein expression. Cntrl, non-treated; H/R 2 h, hypoxia followed by 2 h of reoxygenation. (a) Representative immunoblots. (b) Quantitative data. $*p < 0.01$ when compared with normoxic non-treated samples; $\dagger p < 0.01$ when compared with hypoxia/reoxygenation non-treated samples (ANOVA). Values represent mean ± SD; $n = 3$.

Discussion

ASA and its metabolites have been shown to be protective against cellular injury in non-neuronal systems by inhibiting cyclooxygenases and iNOS or by preventing NF-κB nuclear translocation (Mitchell *et al*. 1993; Amin *et al*. 1995; Yin *et al*. 1998). More recently, inhibition of the p44/42 MAPK signaling pathway has been proposed to be involved (Pillinger *et al*. 1998). In our rat SC culture model, where ≈70% of the cells are neurons, 20% astrocytes and 5% microglia (Vartiainen *et al*. 1999), significant amounts of neither of the COX enzymes could be detected (Vartiainen *et al*. 2001). In addition, previous results indicate that iNOS does not play a role in our hypoxia/reoxygenation model, and that 3 mmol/L ASA does not significantly inhibit DNA binding activity of NF-κB in normoxia or after hypoxia/reoxygenation (N. Vartiainen *et al.* unpublished). Even though kinase-signaling pathways are implicated as targets of ASA, the present work shows for the first time that ASA regulates Cdk5 kinase activity through modulating both Cdk5 and p35 protein expression. Furthermore, the regulation of Cdk5 activity is linked to the neuroprotection gained by treatment with ASA.

Cdk5, together with its neuron-specific activator p35, are needed for neurite outgrowth in the normal brain (Ohshima *et al*. 1996). However, a prolonged activation of Cdk5 in the cytoplasm disrupts the cytoskeleton and promotes apoptotic neuronal death of cultured primary neurons (Patrick *et al*. 1999). This neurotoxic activation can be triggered by ischemia and hypoxic stress in a calcium and calpain activation-dependent manner (Lee *et al*. 2000). Our finding that Cdk5 activity is decreased after hypoxia and up to 2 h of reoxygenation is in disagreement with previous studies. However, cleavage of p35 activator to p25, which is thought to be responsible for the sustained and toxic activation of Cdk5, has been previously detected occurring 4 h after reperfusion *in vivo* or after 5 h of oxidative stress *in vitro* (Lee *et al*. 2000), whereas in the present study p25 immunoreactivity was not detectable after hypoxia or at 2 h of reoxygenation. In our hypoxia/reoxygenation model the major neuronal loss occurs as early as 2–4 h after the onset of reoxygenation. It is probable that p25-induced sustained activation of Cdk5 does not play a role in the acute neuronal death observed in SC cultures after reperfusion/reoxygenation. Instead, down-regulation of Cdk5 activity during reoxygenation is detrimental and mediates early neuronal death.

Pretreatment with ASA enhanced phosphorylation of NFs when studied after 4 h of reoxygenation, and this increased phosphorylation was inhibited by administration of Cdk5 inhibitors, indicating that the rise in Cdk5 activity achieved by ASA contributes to NF phophorylation. This observation is of interest, because phosphorylated NFs have been reported to be more resistant than non-phosphorylated NFs against calpain cleavage (Pant 1988), which takes place after activation of NMDA receptors, resulting in disruption of NFs and neuronal structure. It is possible that Cdk5-mediated NF phosphorylation is one of the mechanisms by which ASA renders neurons more resistant to hypoxia/reperfusion injury.

The mechanism of how ASA prevents the loss of Cdk5 protein and activity (as well as p35 protein) during early reoxygenation, remains to be studied. This is unlikely to involve p35 cleavage to p25. Cdk5/p35 kinase activity is regulated by a tyrosine kinase pathway (Zukerberg *et al*. 2000), casein kinase I, some unknown kinase pathways and phosphatases, all of which could be potentially influenced by ASA. Yet another mechanism could be reduction of Cdk5 protein degradation by inhibition of proteases, because ASA pre-treated cells were more resistant than non-treated cells against reoxygenation-induced loss of Cdk5 protein. It is possible that ASA directly or indirectly also regulates activators other than p35, which include casein kinase II and some uncharacterized kinases. What ever the mechanism is, it seems not to be mediated by inhibition of COX or iNOS enzymes or NF-κB, as demonstrated by our previous studies, thus suggesting a novel therapeutic action of ASA.

Dysregulation of Cdk5 may play a role in several brain diseases, such as Alzheimer's disease (Patrick *et al*. 1999), Parkinson's disease (Neystat *et al*. 2001), amyotrophic lateral sclerosis (Nguyen *et al*. 2001) and stroke (Lee *et al*. 2000). Hayashi *et al*. (1999) demonstrated increased immunoreactivity of both Cdk5 and p35 after transient focal brain ischemia followed by 1 h of reperfusion in the rat. The immunoreactivity decreased at 3 h in the core area, while the cells at the boundary area remained positive for the antibodies used. As demonstrated in this study, the restored activity of Cdk5 may be linked to neuronal survival also in the *in vivo* model used by Hayashi *et al*. (1999). Further support of this hypothesis was gained by our results where Cdk5 and p35 protein expression correlated well with the Cdk5 kinase activity at the 2-h reoxygenation time-point.

In conclusion, ASA pre-treatment induced tolerance in the rat SC neurons against hypoxia/reoxygenation damage in culture. This tolerance involved regulation of both Cdk5 protein expression and kinase activity as well as p35 protein expression. Considering that ASA protects against focal brain ischemia in the rat (Khayyam *et al*. 1999) and is a major preventive agent against stroke in humans, we hypothesize that ASA-induced chemical pre-conditioning contributes to the beneficial effects of ASA in stroke. In addition, maintenance of Cdk5 activity may be crucial for cellular survival through the acute phase of hypoxia/reoxygenation type injury in the brain and peripheral tissues.

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